



UNIVERSITI PUTRA MALAYSIA

**EXTENSION OF VASE LIFE OF CUT ORCHIDS USING OZONATED WATER AND
1-METHYLCYCLOPROPENE**

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**EXTENSION OF VASE LIFE OF CUT ORCHIDS USING OZONATED
WATER AND 1-METHYLCYCLOPROPENE**

By

PARVIZ ALMASI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

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DEDICATION

This thesis is dedicated to all I love specially

To the soul of my parents, my beloved mother and father

In the heaven who regretfully did not live to see this work.

To my beloved wife Nayier, my sons, Parsa and Amin

For the unconditional patience, love and support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EXTENSION OF VASE LIFE OF CUT ORCHIDS USING OZONATED WATER AND 1-METHYLCYCLOPROPENE

By

PARVIZ ALMASI

May 2013

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Faculty: Agriculture

Orchid industry has a particular situation within ornamental plants in Malaysia. As, 46% of total floriculture industry was occupied by orchid flowers in 2011. Most cut orchid flowers are ethylene sensitive in the spite of having acceptable longevity and postharvest life. When they are packed for export they show ethylene injury symptoms such as bud and floret abscission and short vase life. 1-MCP can control ethylene production and action and probably reduces the ethylene sensitivity. Another possible reason for the short span life may be microbial blockage in xylem conduits. Aqueous ozone can be a good candidate for improving the water conductivity in cut stems by its microbial growth inhibition effect.

1-methylcyclopropane (1-MCP) and aqueous ozone were used for controlling ethylene sensitivity and inhibition of xylem blockage in two ethylene-sensitive cut orchids, *Dendrobium* 'Darren Glory' and *Mokara* 'Calypso Jumbo'. Three experiments were conducted at Department of Crop science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor Darul Ehsan.

In experiment one, inflorescences of 12 cut orchid hybrids consisted of six cut *Dendrobium* hybrids (*Dendrobium* 'Darren Glory', *D.* 'Sonia Red', *D.* 'Sonia White', *D.* 'Sonia Bom', *D.* 'Shavin White' and *D.* 'Jacqueline Concert' x *D.* 'Rinappa') and six *Mokara* hybrids (*Mokara* 'Calypso Jumbo', *M.* 'Chao Praya Classic', *M.* 'Citi Gold', *M.* ' Boy Blue', *M.* 'Red' and *M.* 'Chak Kuan Blue') were determined for their ethylene sensitivity levels. Inflorescences were treated with 0 and 10 $\mu\text{L/L}$ ethylene gas for 24 hours in 79 L chambers. The chambers were aerated and stems of flowers were trimmed and placed in PE bags containing vase solution [distilled water + 250 mg/L 8-hydroxyquinoline citrate (8-HQC) + 150 mg/L citric acid + 4% sucrose, pH=3.5]. Weight loss, vase life, ethylene production and anthocyanin content and expression of ACC synthase and ACC oxidase genes of florets were determined. Results showed that exposure to ethylene caused reduction of vase life tremendously. This was manifested by epinasty, premature wilting and abscission in florets and buds for all hybrids. Weight loss and anthocyanin degradations increased when flowers were exposed to ethylene. After exposure to ethylene, higher expressions of both ACS and ACO genes were found in fully open florets. However, different hybrids showed distinct responses to ethylene sensitivities and degrees of deterioration. Therefore, they can be categorized into two groups, sensitive and less sensitive. *Dendrobium* 'Darren Glory', *D.* 'Jaquelyne Concert' x *D.* 'Rinappa', *Mokara* 'Calypso Jumbo', *M.* 'Chao Praya Classic'(MCPC) and *M.* 'Red' exhibited the utmost epinasty, weight loss and anthocyanin content degradation in sepals and petals thus, can be considered as sensitive group while the rest were less sensitive.

In the second experiment, the inflorescences of two very sensitive (*Dendrobium* 'Darren Glory' and *Mokara* 'Calypso Jumbo') and two less sensitive (D. 'Sonia Bom' and M. 'Citi Gold') hybrids that were determined in experiment first, were studied. Inflorescences were divided into two groups and placed in two 79 L chambers. For fumigation of 0 and 300 nL/L 1-MCP, a small vial containing 37.92 mg Ethylbloc was taped to the one chamber's internal wall. Then, 190 μ L/L deionized water was added to the vial. Both chambers were immediately sealed for 4 hours. Then inflorescences of each chamber divided into two subgroups and after labeling the four 39.5 L chambers inflorescences were placed inside them gently. The chambers were hermetically-sealed. Then, two separate 1 μ L/L ethylene gas, balanced with nitrogen, were injected into the one chamber of each subgroup. After 24 hours the chambers were opened and each inflorescence's basal stem was trimmed to 12 cm from the first open floret. Then, each inflorescence was placed in the bottle with a cylindrical polyethylene bag containing 60 ml distilled water and kept in the laboratory at means temperature, relative humidity and light intensity of 25 °C, 78% and 6.57 μ mol/m²/s, respectively. The results showed that weight loss in all four hybrids after 1-MCP fumigation even with exogenous ethylene treatment remarkably declined except MCG which did not show any variation to the responses of all treatments. Vase life responses of highly sensitive and less sensitive hybrids to the 1-MCP were positive but almost different. Maximum vase life extension was observed in highly sensitive hybrid (MCJ) about 59% with 300 nL/L 1-MCP treatment and minimum extension was occurred in less sensitive hybrid

(MCG) around 39%. So it can be suggested that the effectiveness of 1-MCP on the control of ethylene sensitivity in highly sensitive hybrids was more.

In the third experiment, effects of 1-MCP and aqueous ozone on postharvest quality of two very sensitive cut flowers; *Dendrobium* 'Darren Glory' and *Mokara* 'Calypso Jumbo' were studied.

The inflorescences were treated with 0 and 300 nL/L 1-MCP. After four hours, the chambers were aerated and the stems were trimmed to 12 cm from the basal end of the first open floret. Each inflorescence was put into a bottle containing 250 mL of distilled water (control treatment) and 5.2 mg/L aqueous ozone. Ethylene production, microbial growth, bud opening, 1-aminocyclopropan oxidase activity (ACO) and vase life were measured. Results showed 1-MeCP can control the ethylene production within 5 day after harvest in both studied cut orchids very well and also ethylene production and ACO activity in *D.* 'Darren Glory' was higher than *M.* 'Calypso Jumbo'. Aqueous ozone (5.2 mg/L) declined microbial (bacteria, fungi and yeast) growth when it used as the vase solution and every 24 hours replenished and consequently it was effective on decreasing xylem blockage and the micro-graphs from the xylem showed it clearly.

In the forth experiment effects of 4 concentrations of 1-MCP and optimum concentration of aqueous ozone on postharvest quality of sensitive cut flower; *Mokara* 'Calypso Jumbo' were studied.

The inflorescences were fumigated with 0, 100, 200, 300 and 400 nL/L 1-MCP. After four hours fumigation, the stems were trimmed to 12 cm from the basal end of the first open floret. First 30 inflorescences with and without 1-

MCP fumigation were cut with scalpel into two parts: one had only buds and another one had only open florets. Stem ends were placed in distilled water and ozonized water and then used for ACC content and ACC oxidase activity measurements. Remaining fifty intact inflorescences were placed into the bottles each one, containing 250 mL of distilled water or 3.9 mg/L aqueous ozone based on the combination treatments. Ozonized water was renewed at every 24 hours, but for the rest treatments, vase water was not changed until end of vase life.

The results showed that fumigation of the cut *Mokara* 'Calypso Jumbo' flowers with 100 nL/L 1-MCP, followed by using 3.9 mg/L aqueous ozone as the vase water could be recommended as the best postharvest treatments to maintain quality and extend vase life of MCJ orchid hybrids. In addition, these treatments for MCJ were very effective in extending vase live, increasing water uptake and percentage bud opening and also controlling the bacterial growth in vascular system

Therefore, optimum postharvest treatments for the cut orchid flowers depended on hybrids. Pretreatment of the cut orchid flowers with 100-300 nL/L 1-MCP, followed by using 3.9 mg/L aqueous ozone as the vase solution could be recommended as the optimum postharvest treatments to maintain quality and extend vase life of both the DDG and MCJ orchid hybrids. In addition, 1-MCP pretreatment for MCJ was very effective in extending vase lives and increasing percentage bud opening. Additionally, aqueous ozone can effectively decreased number of bacteria in vase water and vascular vessels. Another important finding of this experiment was the cause of short

vase life in the two cut orchid hybrids. The main reason for the short vase life was due to ethylene sensitivity of the cut flowers and not due to xylem blockage.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**LANJUTAN DAIPADA KEHIDUPAN PASU KERATAN KACUKAN ORKID
MENGUNAKAN AIR OZONATED DAN 1-METHYLCYCLOPROPENE**

Oleh

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Mei 2013

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Industri orkid mempunyai kedudukan penting dalam industri tanaman hiasan di Malaysia. Pada tahun 2011, daripada keseluruhan industri florikultur, 46% adalah daripada tanaman orkid. Kebanyakan keratan bunga orkid adalah sensitif terhadap etilena walaupun mempunyai hayat pasuan dan lepas tuai yang bersesuaian. 1-MCP boleh mengawal pengeluaran dan tindakan etilena yang mungkin dapat mengurangkan kepekaan terhadap etilena. Selain itu, ozon akueus boleh menjadi kaedah yang baik untuk meningkatkan aliran air dalam batang yang disebabkan oleh kurangnya mikrob tersumbat dalam saluran xilem.

Oleh itu, kajian telah dijalankan ke atas keratan orkid hibrid *Dendrobium* spp. dan *Mokara* spp. untuk melihat kepekaan kepada etilena. Seterusnya, 1-methylcyclopropane (1-MCP) dan ozon akueus telah digunakan, masing-masing untuk mengawal kepekaan terhadap etilena dan menghalang xilem tersumbat. Tiga eksperimen telah dijalankan di Makmal Lepas Tuai, Jabatan

Sains Tanaman, Fakulti Pertanian, Universiti Putra Malaysia (UPM),
Serdang, Selangor Darul Ehsan.

Dalam eksperimen pertama, tahap kepekaan terhadap etilena ke atas keratan orkid berbunga pada enam hibrid *Dendrobium* ['Darren Glory' *Dendrobium* (DDG), 'Sonia Red' *D.* (DSR), 'Sonia White' *D.* (DSW), 'Sonia Bom' *D.* (DSB), 'Shavin White' *D.* (DSHW) dan 'Jacqueline Concert' *D.* x'Rinappa' *D.* (DJCR)] dan enam hibrid *Mokara* ['Calypso Jumbo' *Mokara* (MCJ), 'Chao Praya Classic' *M.* (MCPC), 'Chiti Gold' *M.* (MCG), 'Blue Boy' *M.* (MBB), 'Red' *M.* (MR) dan 'Chak Kuan Blue' *M.* (MCKB)] ditentukan. Bunga dirawat dengan 0 dan 10 $\mu\text{L/L}$ gas etilena selama 24 jam di dalam bekas berisipadu 79 L. Bekas tersebut diudarakan dan batang bunga dipotong dan diletakkan di dalam beg PE yang mengandungi larutan pasu [air suling + 250 mg/L 8-hydroxyquinoline citrate (8-HQC) + 150 mg/L asid sitrik + sukrosa 4%, pH = 3.5]. Kehilangan berat, hayat pasuan, pengeluaran etilena dan kandungan antosianin dan ekspresi gen asid sintase 1-aminocyclopropane-1-karboksilik (ACS) dan oksidase (ACO) bunga ditentukan. Keputusan menunjukkan bahawa pendedahan kepada etilena menyebabkan pengurangan ketara hayat pasuan. Ini telah ditunjukkan oleh epinasti, layu pramatang dan keguguran bunga serta tunas bagi semua kacukan. Kehilangan berat dan degradasi antosianin meningkat apabila bunga terdedah kepada etilena. Selepas pendedahan kepada etilena, ekspresi gen ACS dan ACO lebih tinggi pada bunga yang terbuka sepenuhnya. Walau bagaimanapun, kacukan yang berbeza menunjukkan tindakbalas yang berlainan kepada etilena dan darjah kemerosotan. Oleh itu, mereka boleh

dikategorikan kepada dua kumpulan, sensitif dan kurang sensitif. DDG, DJCR, MCJ. MCPC dan MR menunjukkan epinasti, kehilangan berat dan degradasi kandungan antosianin dalam sepal dan kelopak yang tinggi, menjadikan ia boleh dianggap sebagai kumpulan yang sensitif manakala selebihnya adalah kurang sensitif.

Dalam eksperimen kedua, dua hibrid bunga sensitif (DDG dan MCJ) dan kurang sensitif (DSB dan MCG) yang telah ditentukan dalam eksperimen pertama telah dilakukan kajian lanjut. Bunga telah dibahagikan kepada dua kumpulan dan diletakkan dalam dua bekas berisipadu 79 L. Untuk pendedahan kepada 0 dan 300 nL/L 1-MCP, botol kecil yang mengandungi 37.92 mg Ethylbloc dilekatkan pada dinding bahagian dalam bekas. Kemudian, 190 μ L/L air dinyah ion ditambah kepada botol tersebut. Kedua-dua bekas segera ditutup selama empat jam. Kemudian, bunga dari setiap bekas dibahagikan kepada dua kumpulan kecil dan diletakkan dalam dua bekas 39.5 L yang telah dilabel. Bekas ditutup rapat. Kemudian, 1 μ L/L gas etilena yang diseimbangkan dengan nitrogen disuntik dalam bekas tersebut. Selepas 24 jam, bekas dibuka dan setiap batang bunga dipotong sehingga 12 cm dari bunga kecil terbuka pertama. Seterusnya, setiap batang bunga diletakkan dalam botol berisi satu beg polietilena berbentuk silinder yang mengandungi 60 ml air suling dan disimpan pada suhu makmal, 25 °C, kelembapan relatif, 78% dan keamatan cahaya 6.57 μ mol/m²/s. Hasil kajian menunjukkan pengurangan berat bagi tiga daripada empat kacukan menurun dengan ketara selepas rawatan 1-MCP, walaupun telah didedahkan kepada etilena secara luaran, kecuali untuk MCG yang tidak menunjukkan sebarang perubahan sebagai tindakbalas kepada semua rawatan. Hayat pasuan juga

dilanjutkan pada tahap yang berbeza. Lanjutan hayat pasuan maksima diperhatikan dalam hibrid sensitif, MCJ dan lanjutan minima dalam hibrid kurang sensitif, MCG, dengan masing-masing kira-kira 59 dan 39% hari tambahan, dalam larutan pasu dengan 300 nL/L rawatan 1-MCP. Bertepatan dengan 1-MCP yang dikenali sebagai anti-etilena, ia boleh dicadangkan bahawa apabila etilena berada dalam kawalan, hayat pasuan boleh dilanjutkan pada tahap yang berbeza bergantung kepada jenis hibrid.

Dalam eksperimen ketiga, kesan 1-MCP dan ozon akueus kepada kualiti lepas tuai dua hibrid bunga sensitif, DDG dan MCJ dikaji. Bunga dirawat dengan 0 dan 300 nL/L 1-MCP. Selepas empat jam, bekas diudarakan dan hujung batang bunga dipotong 12 cm dari bunga kecil terbuka pertama. Setiap batang bunga dimasukkan ke dalam botol yang mengandungi 250 mL air suling (rawatan kawalan) dan 5.2 mg/L ozon akueus. Pengeluaran etilena, pertumbuhan mikrob, pembukaan putik, aktiviti 1-aminocyclopropane oxidase (ACO) dan hayat pasu diukur. Keputusan menunjukkan bahawa 1-MCP boleh mengawal pengeluaran etilena dalam masa 5 hari selepas penuaian bagi kedua-dua hibrid. Walau bagaimanapun, pengeluaran etilena dan aktiviti ACO dalam DDG adalah lebih tinggi daripada MCJ. Ozon akueus (5.2 mg/L) yang diisi semula setiap hari berjaya mengurangkan pertumbuhan mikrob (bakteria, kulat dan yis) dan seterusnya, mengawal xilem tersumbat yang mana jelas ditunjukkan dari graf mikro.

Oleh itu, rawatan lepas tuai optima untuk keratan orkid yang sensitif etilena bergantung pada kacukan. Rawatan awal keratan bunga orkid dengan 300

nL/L 1-MCP diikuti dengan 5.2 mg/L ozon akueus sebagai larutan pasu boleh disyorkan sebagai rawatan lepas tuai pilihan untuk mengekalkan kualiti dan memanjangkan hayat pasu kedua-dua hibrid orkid, DDG dan MCJ. Di samping itu, prarawatan 1-MCP untuk MCJ adalah sangat berkesan dalam memanjangkan hayat pasu dan meningkatkan peratusan pembukaan putik.



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I certify that a Thesis Examination Committee has met on 27 May 2013 to conduct the final examination of Parviz Almasi on his thesis entitled "Extension of Vase Life of Cut Orchids using Ozonated Water and 1-Methylcyclopropene" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

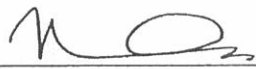
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